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Exposure to restraint stress resulted in antagonism of the analgesic effect of administered morphine in adult male rats. This antagonism of morphine-induced analgesia by restraint stress was not affected by adrenalectomy one day prior to testing, suggesting that stress-induced secretion of corticosteroids is not critical to this antagonism. In addition, parenteral administration of exogenous adrenocorticotropin (ACTH) mimicked the effect of stress in antagonizing morphine's analgesic efficacy. These results support the hypothesis that ACTH is an endogenous opiate antagonist involved in modulating pain sensitivity.

Adrenocorticotropin	Morphine-induced analgesia	Stress
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1. Introduction

We have shown previously, through classical endocrine manipulations, that altering the endogenous circulating level of ACTH results in changes in sensitivity to pain (Heybach and Vernikos, 1978). Hypophysectomy reduces pain sensitivity in the rat and in man (Luft and Olivecrona, 1955), and this effect is reversed in the rat by the intracerebroventricular (ICV) administration of the synthetic opiate antagonist naloxone (Heybach and Vernikos, in press). These observations promoted us to suggest that pituitary ACTH acts as an endogenous hyperalgesic factor and opiate antagonist (Heybach and Vernikos, 1978; 1981). Evidence in favor of this suggestion were the findings that the ICV injection of crude ACTH, ACTH₁₋₂₄, or α -MSH resulted in increased sensitivity to a painful thermal stimulus in the rat and that the peripheral administration of these peptides antagonized the analgesia in morphine pretreated rats (Gispen et al., 1976; Paroli, 1967; Heybach and Vernikos, 1981). In the course of these experiments we also observed that the stress of a saline injection in our control animals also reduced the analgesic effect of morphine pretreatment though to a much lesser extent than peptide treatment.

It is well known that the pituitary gland synthesizes and secretes ACTH in response to stressful stimuli (Vernikos-Danellis, 1963). The present study was therefore undertaken to determine the effect of stress-induced release of endogenous ACTH on the analgesic action of morphine and on pain sensitivity in general. The results indicate that restraint stress reduces the efficacy of administered morphine in reducing the sensitivity to a painful thermal stimulus. This antagonism occurs in adrenalectomized animals as well as in normal animals and can be reproduced by the administration of exogenous ACTH.

2. Materials and methods

Male Sprague-Dawley rats (Simonsen Laboratories, Gilroy, Calif.) weighing 200 ± 10 g were used throughout these experiments. They were housed 4-5 per cage in plastic-top loading cages with woodchip bedding and maintained on a 12-h light/dark cycle (lights on at 0700 h) in a vivarium with temperature maintained at $23 \pm 3^\circ\text{C}$. Rats had ad libitum access to standard laboratory chow and tap water. Groups of at least 8 rats were used in each experimental condition.

Drugs used and their suppliers were: ACTH from Armour Labs, Kankakee, Illinois; morphine sulphate from Endo Labs, Garden City, New York. Doses of morphine were calculated as the free base.

Adrenalectomies were performed via the dorsal approach under ether anesthesia. After surgery, adrenalectomized rats were provided with 0.9% saline instead of tap water. Following all experimental procedures, adrenalectomized rats were sacrificed and examined for completeness of adrenalectomy.

The apparatus used to assess pain sensitivity has been described in detail previously (Heybach and Vernikos, 1978). The procedure for estimating pain sensitivity was standardized for all experiments. All animals were initially allowed three 90-sec sessions, one session per day for 3 consecutive days, with the temperature of the grid floor maintained at $23 \pm 1^\circ\text{C}$. This procedure familiarized the rats with handling and allowed for habituation to the novelty of the apparatus. To initiate a testing session, the rat was placed on the grid floor of the apparatus with the temperature maintained at $55 \pm 1^\circ\text{C}$ and a stop watch was started simultaneously. Two responses were then recorded: the latency to lift from the floor and lick one paw (i.e., paw lick latency) and, subsequently, the latency to show a jump response consisting of

vigorously lifting both hind paws off the grid floor simultaneously (i.e., jump latency). The paw lick and jump latencies were used as indices of the sensitivity to the painful stimulus. The experimenter was not aware of the pretreatment of the rats during testing. If neither a paw lick nor jump response occurred within 90 sec the test was terminated and a latency of 90 sec was recorded for each response. All testing was carried out between 0800-1200 h.

Restraint stress consisted of placing the rat in a semicylindrical plexiglass device that comfortably restricted movement. Animals were injected intraperitoneally (i.p.) with morphine immediately upon removal from the restraint device and tested for pain sensitivity at 10, 20, and 30 min later. In one experiment rats were also tested 60 min after the morphine injection to determine the duration of its effect.

3. Results

Figure 1 shows the results of an experiment investigating the effect of the duration of the restraint stress on the analgesic efficacy of morphine. Three different dose levels of morphine (2.5, 5.0, and 10 mg/kg) were used. They were injected using a Latin square design to minimize errors due to experimental bias. Restraint stress alone for 15, 30, or 60 min had no effect on the sensitivity to this painful thermal stimulus. The three doses of morphine resulted in a dose-related degree of analgesia. Exposure to restraint stress for 15 min completely prevented the analgesic effect of 2.5 mg/kg morphine and significantly reduced that of 5.0 mg/kg, whereas it had no effect on the 10 mg/kg dose. Thirty minutes of restraint also significantly reduced the analgesic action of both the 2.5 and 5.0 mg/kg doses, having little effect on the largest dose. On the other hand, 60 min of restraint had no effect on

Fig. 1

the action of either of the lower doses of morphine, but significantly potentiated the 10 mg/kg dose.

Figure 2 shows the time course of the analgesia produced by the three doses of morphine as compared to saline-injected controls and the effect of 15 min of restraint stress on that response as measured by jump latencies. The analgesic effect of morphine was evident as early as 10 min after its injection and with the two higher doses continued to increase at 30 min. Fifteen minutes of restraint stress effectively antagonized the 2.5 and 5.0 mg/kg doses at all time intervals tested. The data show that although stress effectively antagonized the 10.0 mg/kg dose when the animals were tested at 10 and 20 min, the effect was transient and at 30 min after the morphine injection this large dose was as effective in the stressed as in the non-stressed animals.

To determine whether stress-induced adrenocortical secretion was involved in this antagonism of the analgesic effect of morphine, animals that had been adrenalectomized one day previously were exposed to 15 min of restraint or no restraint and then given 5.0 mg/kg morphine. Figure 3 shows that the absence of the adrenals did not affect the ability of stress to reduce the analgesic action of this dose of morphine.

Figure 4 shows the comparison of the effect of 15 min of restraint or of a single dose of ACTH (50 uU, i.p.) administered 15 min prior to the injection of morphine (5.0 mg/kg). Animals were tested 30 min later. It can be seen that this dose of ACTH is as effective as 15 min of restraint in reducing the analgesic action of morphine.

4. Discussion

The antagonism of the analgesic effect of morphine by prior exposure of an animal to 15 or 30 min of stress adds additional support to the hypothesis that an endogenous opiate antagonist exists and that this substance may be ACTH or an ACTH-like peptide from the pituitary. During restraint stress, as with other stressful stimuli, ACTH is secreted very rapidly with maximal levels occurring within 2.5 min after the stress begins; the level then falls gradually for the next 60 min, but remains above control values (Sakellaris and Vernikos-Danellis, 1975).

Plasma corticosterone reaches maximal levels within 10 min after restraint begins and is sustained for the remaining 60 min. However, the persistence of the stress-induced antagonism of morphine analgesia in the adrenalectomized animals suggests that the secretions of the adrenal cortex are not involved in this effect. However, the potentiation of the analgesia produced by the largest dose of morphine by prior exposure to 60 min of restraint may be partly attributable to the high circulating levels of corticosteroids. Holaday et al. (1979) obtained evidence that corticosteroids may increase the parenteral potency of morphine by increasing its bioavailability through inhibition of its metabolism. Even more likely is the possibility that endogenous opiate activity may be increased relative to ACTH at 60 min and may result in the potentiation observed at that time of the effect of the largest dose of morphine. Although it has been reported that pituitary endorphin is secreted concomitantly with ACTH in response to acute stress (Guillemin et al., 1977), the dynamics of its response to a more sustained stress, such as restraint, have not been described. Such description has also been hampered by the problem of specificity of the antibodies used in the radioimmunoassay of endorphins

in the early phases of this research and by the lack of correlation between immunoreactivity and opiate activity (Li et al., 1977). Nevertheless, potentiation of morphine analgesia by more long-lasting stress, as seen in this study, may involve mechanisms that are akin to the stress-induced analgesia reported by others (Akil et al., 1976). In our study we never observed an analgesic effect of this form of restraint stress alone.

Fifteen minutes of restraint was as effective in reducing the analgesic efficacy of morphine as 50 uU of ACTH (i.p.) administered 15 min earlier. Thus, it would appear that the effect of short periods of stress in antagonizing morphine-induced analgesia may be mediated by this pituitary peptide. ACTH is effective in antagonizing morphine whether it is given before the opiate as in this study, or after the opiate (Heybach and Vernikos, 1981). It is also effective in this respect at doses that do not in themselves produce increased sensitivity to pain.

ACTH and ACTH-like peptides have been shown to antagonize morphine (Terenius et al., 1975) and β -endorphin binding (Akil et al., 1980) to preparations of rat brain homogenates in vitro. Therefore, from this data and that of our studies (Heybach and Vernikos, 1978; in press) it would appear that the pituitary is the source of an endogenous opiate antagonist and hyperalgesic factor, probably ACTH or an ACTH-like peptide secreted and regulated in exactly the same way as ACTH, and that its action is via a direct effect on central nervous system opiate receptors.

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Fig. 1. The effect of restraint stress for 15, 30, or 60 min on the analgesic action of various doses of morphine (2.5, 5, and 10 mg/kg; i.p.). Analgesia was measured as the effect on paw lick (A) and jump (B) latencies, 30 min after the administration of morphine.

Morphine 2.5 mg/kg

15 min stress vs no stress (NS)

Paw lick: $F(1, 23) = 16.1, p < 0.005$

Jump: $F(1, 23) = 36.8, p < 0.001$

30 min stress vs no stress (NS)

Paw lick: $F(1, 21) = 6.27, p < 0.05$

Jump: $F(1, 21) = 12.2, p < 0.01$

Morphine 5.0 mg/kg

15 min stress vs no stress (NS)

Paw lick: $F(1, 25) = 13.2, p < 0.005$

Jump: NS

30 min stress vs no stress (NS)

Paw lick: $F(1, 22) = 24.2, p < 0.001$

Jump: NS

Fig. 2. Time course of the effect of three different doses of morphine (2.5, 5.0, and 10.0 mg/kg) administered i.p. or saline in unstressed animals or in animals subjected to 15 min restraint. Analgesia was measured as the effect on jump latencies.

Fig. 3. The lack of effects of adrenalectomy on the antagonism of morphine analgesia by restraint stress.

Fig. 4. The effect of treatment with ACTH (Armour; 50 uU i.p. 15 min previously) or restraint stress for 15 min on paw lick and jump latencies 30 min after morphine pretreatment (5 mg/kg; i.p.). Each bar represents the mean \pm S.E. of the mean.

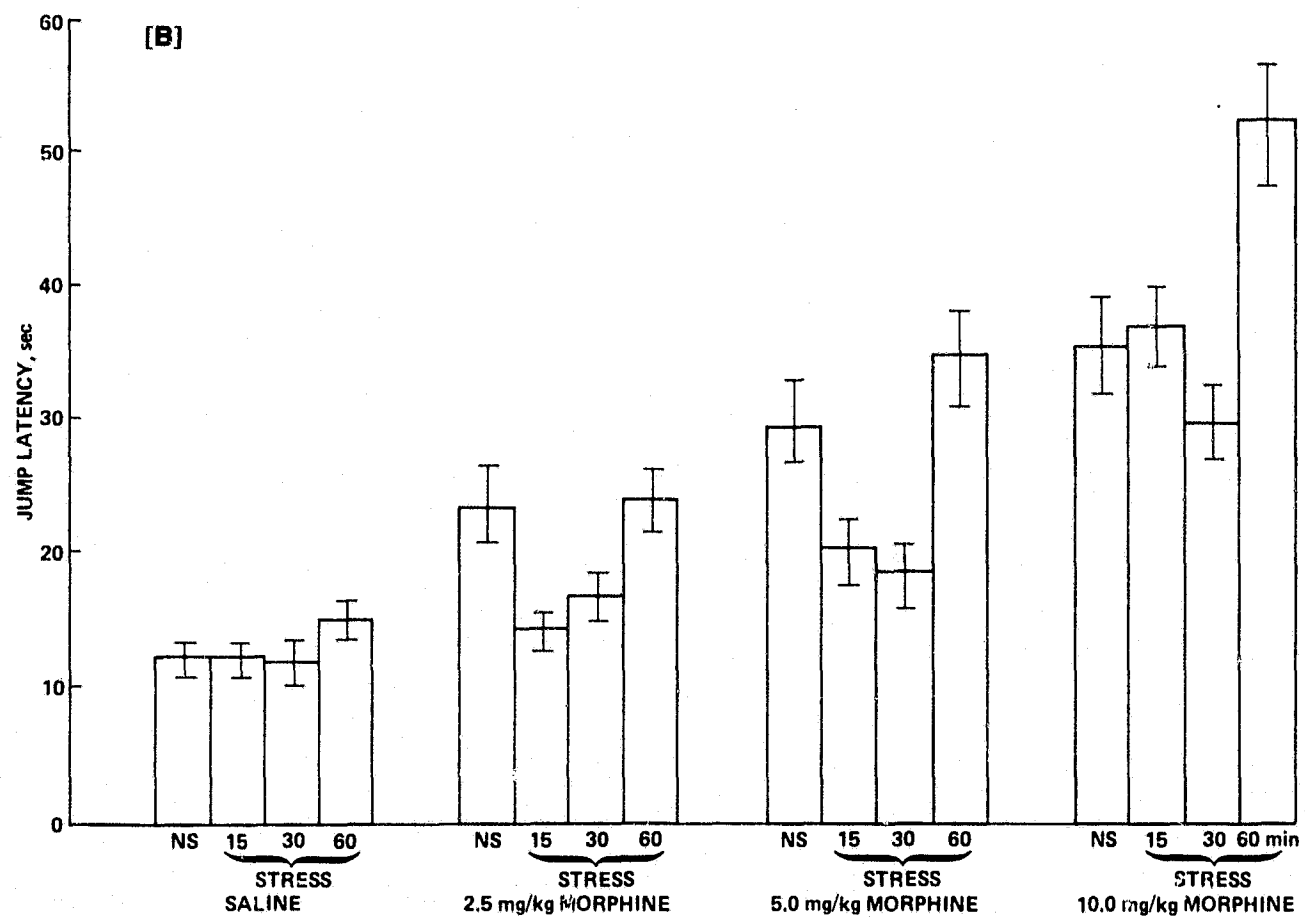
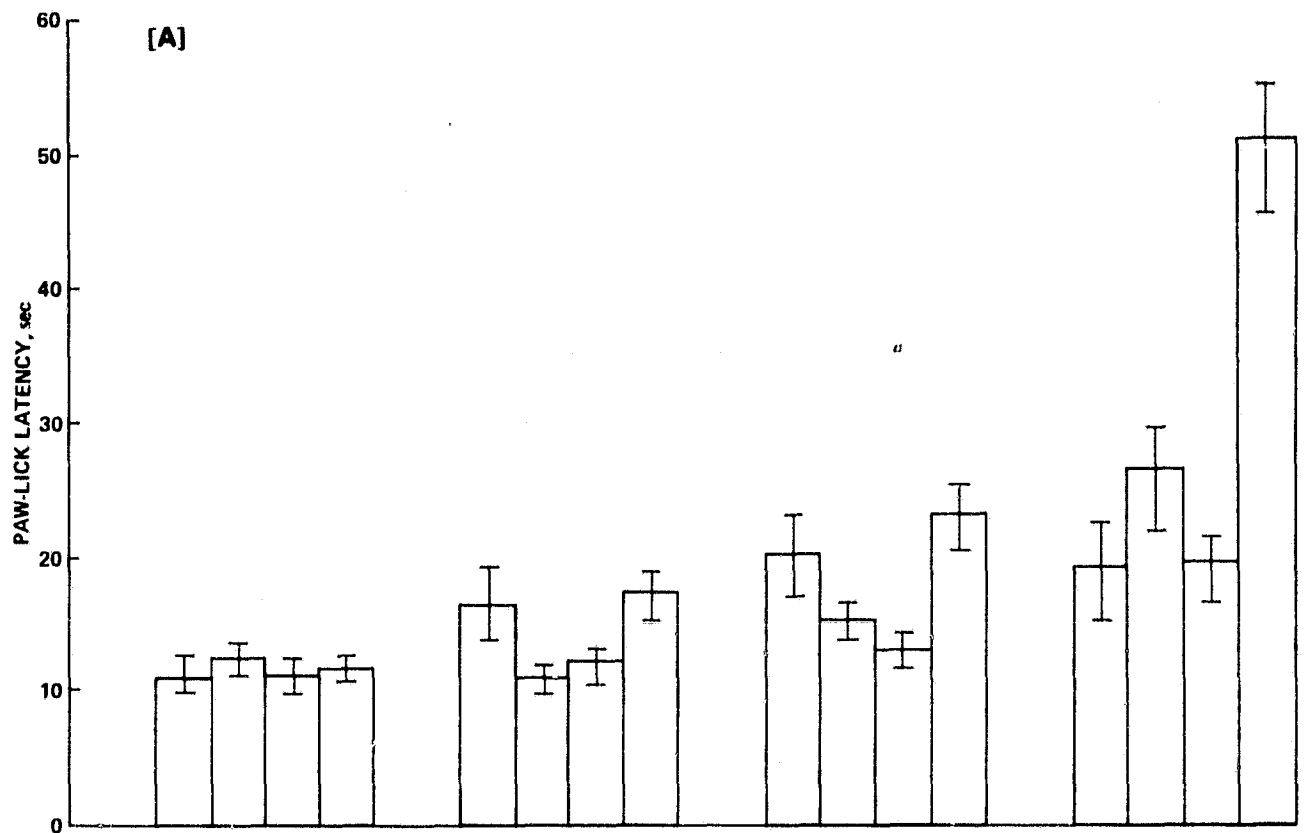


Fig. 1

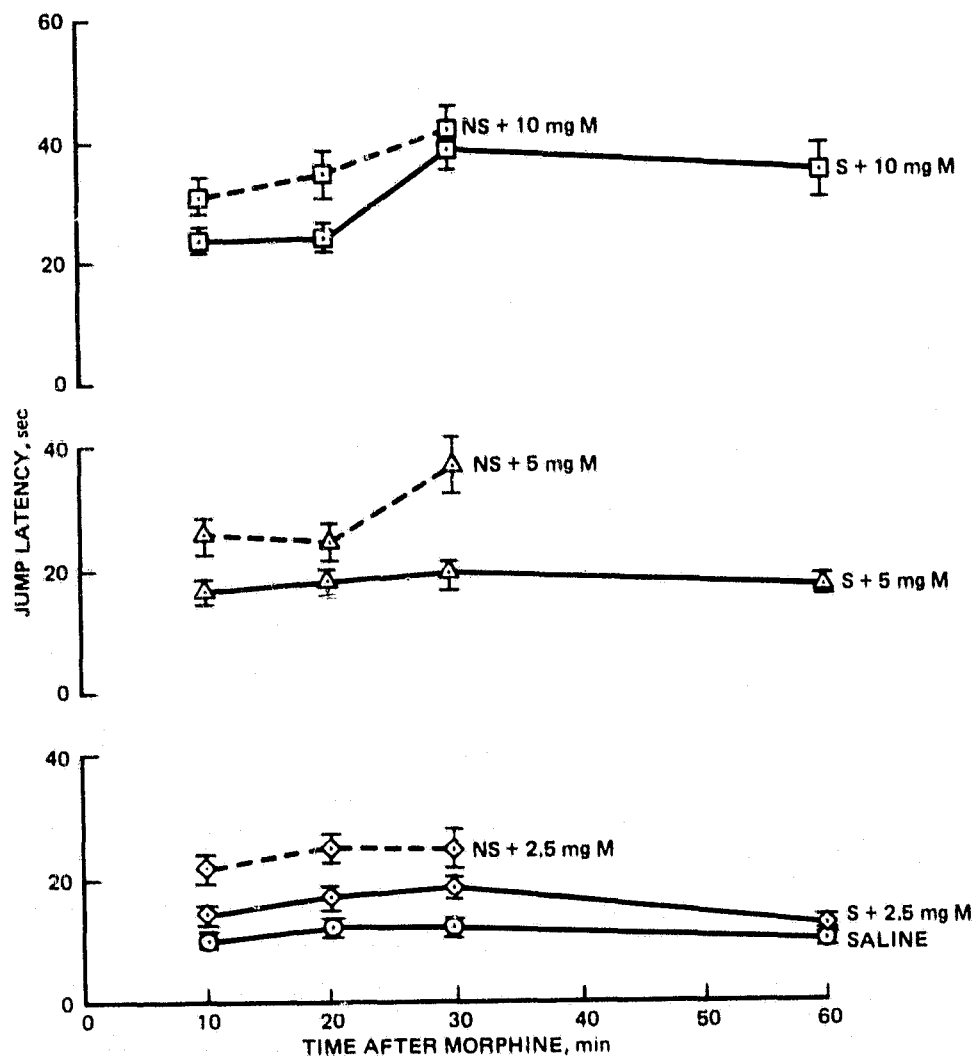


Fig. 2

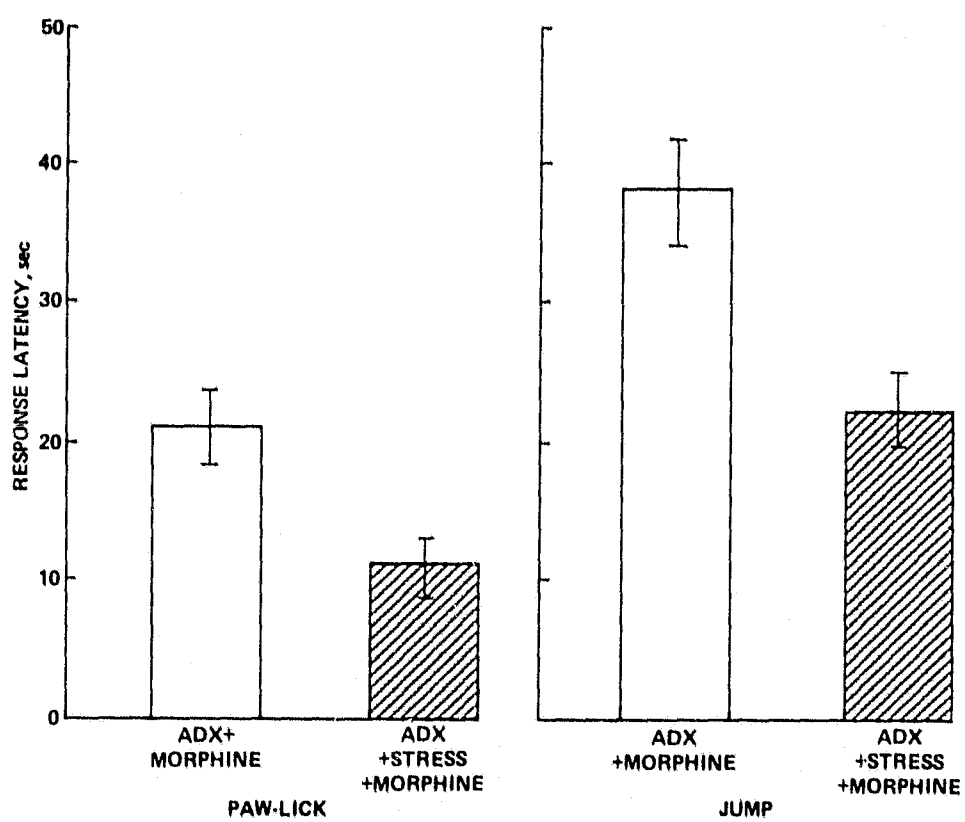


Fig. 3

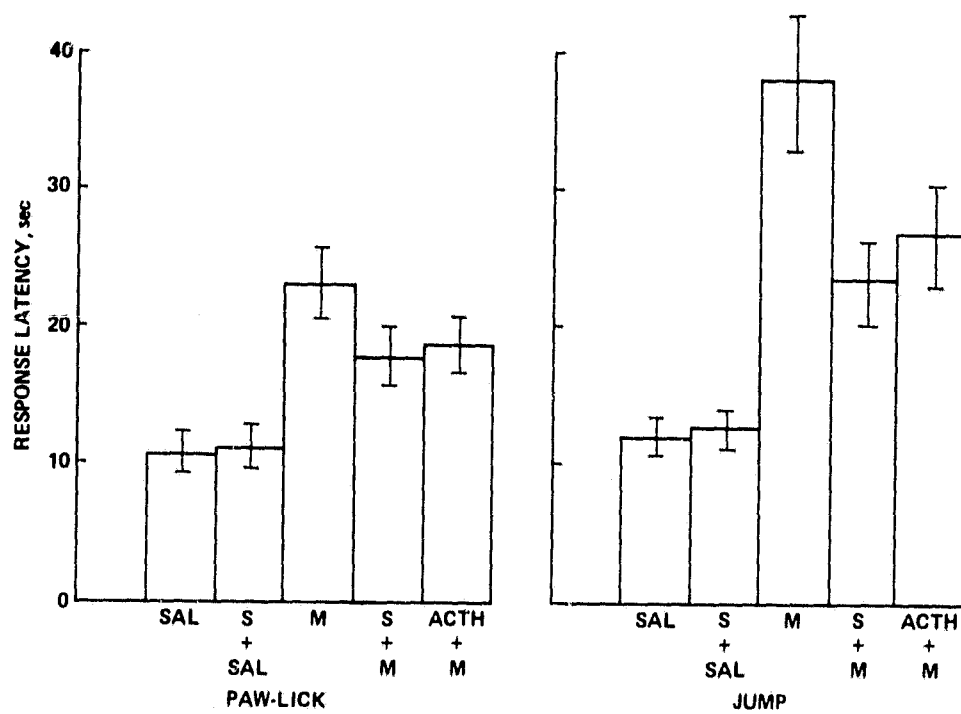


Fig. 4